The F-Like Protein Ac23 Enhances the Infectivity of the Budded Virus of gp64-Null Autographa californica Multinucleocapsid Nucleopolyhedrovirus Pseudotyped with Baculovirus Envelope Fusion Protein F

Manli Wang,1,3‡ Ying Tan,1,3‡ Feifei Yin,1,3 Fei Deng,1 Just M. Vlak,2 Zhihong Hu,1 and Hualin Wang1*

State Key Laboratory of Virology and Joint Laboratory of Invertebrate Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, People’s Republic of China; Laboratory of Virology, Wageningen University, 6709 PD Wageningen, The Netherlands; and Graduate School of the Chinese Academy of Sciences, Beijing 100039, People’s Republic of China

Received 7 April 2008/Accepted 16 July 2008

The GP64 and F proteins were previously identified as the sole functional envelope fusion proteins in Baculoviridae. F-like proteins, present only in group I nucleopolyhedroviruses (NPVs), are remnant, nonfunctional F proteins. In this report, we describe the effect of the presence or absence of the F-like protein Ac23 in a gp64-null Autographa californica multinucleocapsid NPV pseudotyped with the F protein from Spodoptera exigua multicapsid NPV (SeF). We found that the presence of Ac23 elevates the infectivity of the pseudotyped virus. This is in contrast to the results of Lung et al. (J. Virol. 76:5729-5736, 2002), who found no such effect. The possible reasons for the differing results are discussed.

The entry of enveloped viruses into host cells is usually mediated by one or more of their envelope proteins. Two types of envelope fusion proteins, GP64 and F, were identified in budded viruses (BV) of group I and group II nucleopolyhedroviruses (NPVs), respectively (3, 9, 14). These proteins are responsible for virus binding to cell receptors, for mediating the fusion of viral and endosomal membranes, and for efficient budding and egress (1, 2, 5, 6, 11). They are considered to be the only functional membrane fusion proteins within the Baculoviridae family.

Apart from GP64, group I NPVs also contain F-like proteins, such as those encoded by Ac23 from Autographa californica multinucleocapsid NPV (AcMNPV) (7) and Op21 from Orgyia pseudotsugata MNPV (10). These F-like proteins coexist with GP64 on the surface of BVs (10) and are thought to be remnants of a functional F. The F-like proteins are structurally different from F proteins in that the former lack a furin cleavage site and thus cannot function as fusion proteins (10). Lung et al. (7) have shown that Ac23 is not essential for AcMNPV infection, propagation, and BV production in cell culture but that it is a viral pathogenicity factor. Insects infected with AcMNPV lacking Ac23 kill infected insect larvae in a longer time (7). However, since the F-like proteins are present in BVs, we hypothesize that the proteins may have function at the cellular level. Therefore, in the current study, the function of Ac23 was investigated in the absence of GP64 but in the presence of a functional but less-fusogenic F protein from group II NPVs (SeF). We observed that a deletion mutant of AcMNPV lacking both GP64 and Ac23 but containing SeF (vAcgp64−/Ac23−/SeF) produced significantly fewer infectious BVs than wild-type AcMNPV. This system appeared suitable to investigate possible auxiliary functions of Ac23 in BV infection.

To investigate the function of Ac23 in the absence of GP64, recombinant AcMNPV bacmids were generated (Fig. 1), namely, the “wild type” control bAc-egfp, with egfp transposed into the polyhedrin locus; AcMNPV lacking gp64 (bAcgp64−/Ac23−) and AcMNPV lacking both gp64 and Ac23 (bAcgp64−/Ac23−); both mutants carrying SeF (bAcgp64−/SeF and bAcgp64−/Ac23−/SeF), with an egfp and a cat cassette in the gp64 locus; and a control bacmid, an AcMNPV rescue bacmid with gp64 and Ac23 reinserted into AcMNPV (bAcgp64−/Ac23−/gp64-Ac23). Details of the construction and verification of the bacmids are in the supplemental material. Recombinant bacmids were transfected into Sf9 cells, and 4 days posttransfection (p.t.), green fluorescence was detected in the cells, indicating successful transfection (Fig. 2A, panels a, b, c, d, e, and f). At 6 days p.t., a supernatant sample was used to infect another batch of Sf9 cells, which were inspected 4 days postinfection (p.i.) for enhanced green fluorescent protein (EGFP) fluorescence.

Bacmids bAcgp64−/Ac23− (Fig. 2A, panel b') and bAcgp64−/Ac23− (Fig. 2A, panel d') did not produce infectious BVs because of the absence of the gp64 gene, which is essential for viral entry and budding, as reported by previous studies (1, 2, 8). However, with the control bacmid bAc-egfp (Fig. 2A, panel a') and the full-reverse bacmid bAcgp64−/Ac23−/gp64-Ac23 (Fig. 2A, panel f'), the transfected cells produced BVs which were able to infect healthy cells. The F-repaired bacmids bAcgp64−/SeF (Fig. 2A, panel c') and bAcgp64−/Ac23−/SeF (Fig. 2A, panel c') were able to produce BVs and generate systemic infections in the Sf9 cell culture that were comparable to those of control bacmid-derived BVs (Fig. 2B). These results are consistent with a previous conclusion that F proteins are functionally analogous to GP64 (6) and that Ac23 is not essential for BV production in the presence of GP64 (7). We now demonstrate that Ac23 is also not essential for BV production when
AcMNPV is pseudotyped with a functional heterologous F protein.

To see to what extent Ac23 contributes to the production of infectious BVs, one-step growth curves of vAc-egfp, vAcgp64- -SeF, and vAcgp64- Ac23- -gp64-Ac23 were made and compared (Fig. 2B). Sf9 cells were infected with the respective viruses, and the supernatants sampled at different time points after infection and tested in an end point dilution assay (4). The BV production curves were very similar for respective lanes (Fig. 3D).

FIG. 1. Genomic structure of recombinant bacmids bAc-egfp, bAcgp64- -SeF, bAcgp64- Ac23- -SeF, bAcgp64- Ac23- -SeF, bAcgp64- -gp64-Ac23 and parental bacmid AcMNPV (bMON14272). Dash-and-dot lines represent the substitution of the corresponding cassettes.

To investigate whether the reduced infectivity of vAcgp64- Ac23- -SeF was due to lower BV yield or lower infectivity, quantitative real-time PCR was performed on the various recombinant viruses, using primers for the amplification of a 340-bp region of the lef-8 gene (GeneID no. 1403882) (the methodology is described in the supplemental material). The quantitative PCR results show that for vAcgp64- Ac23- -SeF, one TCID<sub>50</sub> unit tested by end point dilution assay was equivalent to 10<sup>7</sup> copies of viral genome DNA (Table 1). For the
FIG. 2. Transfection and infection assays of AcMNPV recombinants for viral propagation. (A) Sf9 cells were transfected with bacmids bAc-egfp (a), bAcgp64 (b), bAcgp64-SeF (c), bAcgp64-Ac23 (d), bAcgp64-Ac23-SeF (e), and bAcgp64-Ac23-gp64-Ac23 (f). One microgram of DNA of each recombinant bacmid was transfected into 1 × 10⁶ Sf9 cells in a 35-mm-diameter tissue culture dish by using 12 μl Lipofectin reagent according to the Bac-to-Bac expression system manual (Invitrogen). Six days p.t., supernatants from the transfected cells (panels a, b, c, d, e, and f) were used to infect healthy Sf9 cells (panels a', b', c', d', e', and f'). Levels of infection were recorded by fluorescence at 4 days p.t. or p.i. (B) Results of one-step BV growth curve analyses of vAc-egfp, vAcgp64-SeF, vAcgp64-Ac23-SeF, and vAcgp64-Ac23-gp64-Ac23. Sf9 cells were infected at a multiplicity of infection of 5 TCID₅₀ U per cell for each virus in triplicate. Titers of progeny viruses at various time points p.i. were determined by performing an end-point dilution assay (4). Data points represent the mean titers of the triplicate infections, and error bars represent the standard deviations from the means. BV titers were logarithmically transformed and analyzed by using two-way analysis of variance (SPSS, Inc., 2003) with virus type and time as factors.
control viruses, vAcgp64-Ac23-SeF, vAcgp64-Ac23gp64-Ac23, and vAc-egfp, one TCID<sub>50</sub> unit was equivalent to 10<sup>4</sup> copies of viral genome DNA (Table 1). Therefore, vAc<sup>gp64−Ac23−</sup>-SeF was about 10 times less infectious than the control viruses (F = 33.731; df = 3 and 11; P < 0.01). However, according to the end point dilution assay results, the level of infectious-BV production of vAcgp64-Ac23-SeF was about 100 times lower than the levels for the control viruses, indicating that the BV yield was also reduced.

Taken together, our results indicate that the presence of Ac23 contributes to both the yield and the infectivity of BVs in cell culture. This result is consistent with and provides an explanation for previous findings that Ac23 is a pathogenicity factor in vivo and that the infection of insects by AcMNPV is severely delayed when Ac23 is not present (7). Our findings on the contribution of Ac23 to the infectivity of BVs also suggest that the product of Ac23 contributes to the binding process and may be an accessory protein, which is consistent with indirect evidence obtained by Zhou and Blissard (16). Although our construct required the presence of Ac23 to achieve full levels of infectivity, a construct described by Lung et al. (6) was defective even when Ac23 was present. The major differences

![FIG. 2—Continued.](image1)

![FIG. 3. Results of Western blot analyses of proteins corresponding to the respective viruses. Lanes for all panels are as follows: 1, vAc<sup>gp64−Ac23−</sup>-SeF; 2, vAc<sup>gp64−SeF</sup>; 3, vAc<sup>gp64−Ac23−gp64-Ac23</sup>; and 4, vAc-egfp. Lane M contains the protein markers, with molecular masses indicated on the left. BVs were harvested and purified from the supernatants of infected Sf9 cells at 3 days p.i., and the proteins were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The blots were probed with antibodies as shown above the panels. The anti-GP64 antibody used was monoclonal antibody AcV5 at a 1:1,000 dilution.](image2)
between our construct and the one described in reference 6 are
the type and location of the reporter genes used (EGFP-polycyto-
l亲眼 locus and GUS-gp64 locus, respectively). In addition, SeF
was expressed from both the gp64 and the polyhedrin
promoter in our construct but only from the gp64 promoter
in the construct of Lung et al. (6). Therefore, the levels of SeF
expressed from our construct may have been higher than in
the construct of Lung et al. (6). However, the precise role that
these two constructs remains to be determined.

In summary, the results of our study provide strong support
for the view that the F-like protein Ac23 is a virulence factor
not only in vivo but also in vitro. The F-pseudotyped gp64-null
AcMNPVs used in the current study are considered to be
a useful platform for studying further the function of F-like
proteins in baculovirus infection both in vitro and in vivo.

This work was supported by grants from the National Natural
Science Foundation of China (30470076, 30630002, and 30670078),
the 973 project (2003CB114202), and the PSA project from MOST and
KNAW (2004CB720404).

We thank Xiulian Sun for statistical analysis and Yanfang Zhang for
cell culture.

REFERENCES
protein is sufficient to mediate pH-dependent membrane fusion. J. Virol.
66:6829–6835.
Blissard. 1999. Host cell receptor binding by baculovirus GP64 and kinetics

the cytoplasmic tail domain of the major envelope fusion protein of group II
Pseudotyping Autographa californica multicsapid nucleopolyhedrovirus
(AcmNPV): F proteins from group II NPVs are functionally analogous to
AcmNPV GP64. J. Virol. 76:5729–5736.
fusion protein homolog in the baculovirus Autographa californica multicsapid
nucleopolyhedrovirus, is a viral pathogenicity factor. J. Virol. 77:328–339.
efficient budding of Autographa californica multicsapid nucleopolyhedrovi-
Lymantria dispar nucleopolyhedrovirus envelope fusion protein provides ev-
idence for a phylogenetic division of the baculoviridae. J. Virol. 74:6126–
6133.
a baculovirus-encoded protein that is associated with infected-cell mem-
analysis of a conserved region of the baculovirus envelope fusion protein,
The F protein of Helicoverpa armigera single nucleopolyhedrovirus can be
substituted functionally with its homologue from Spodoptera exigua multiple
and D. Zuidema. 2002. Furin is involved in baculovirus envelope fusion protein
sequence analysis of a gene encoding gp67, an abundant envelope glyco-
protein of the baculovirus Autographa californica nuclear polyhedrosis virus.
californica multiple nucleopolyhedrovirus nucleocapsid assembly is inter-
pre64-null baculovirus virions and enhanced budding mediated by a vesicular

### Table 1. Quantitative PCR for determining viral genome DNA copies compared to viral titers

<table>
<thead>
<tr>
<th>Recombinant virus</th>
<th>Amt of DNA (copies/ml)</th>
<th>Virus titer (TCID50 U/ml)</th>
<th>Viral infectivity (copies/TCID50 U [avg ± SD])</th>
</tr>
</thead>
<tbody>
<tr>
<td>vAc-egfp</td>
<td>6.0 × 10^10</td>
<td>6.3 × 10^6</td>
<td>(1.1 ± 0.1) × 10^4</td>
</tr>
<tr>
<td>vAc gp64-Ac23–gp64-Ac23</td>
<td>1.7 × 10^11</td>
<td>1.0 × 10^7</td>
<td>(1.7 ± 0.7) × 10^4</td>
</tr>
<tr>
<td>vAc gp64-Ac23–SeF</td>
<td>1.9 × 10^11</td>
<td>1.2 × 10^7</td>
<td>(1.6 ± 0.6) × 10^4</td>
</tr>
<tr>
<td>vAc gp64-Ac23–SeF</td>
<td>1.7 × 10^10</td>
<td>1.7 × 10^5</td>
<td>(10.0 ± 2.0) × 10^4</td>
</tr>
</tbody>
</table>

*a* Fresh BVs of vAc-egfp, vAc gp64–Ac23–gp64-Ac23, vAc gp64–Ac23–SeF, and vAc gp64–SeF were harvested 96 h p.i. and tittered by end point dilution assay.

*b* Viral DNAs were extracted, and quantitative PCR was performed to determine genomic DNA copies based on a standard curve.